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EFFECTS OF DISSOLVED LEAD AND COPPER ON THE FRESHWATER PROSOBRANCH *LANISTES CARINATUS*

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ABSTRACT

Lead and copper bioconcentration and toxicity to the freshwater prosobranch *Lanistes carinatus* (Olivier, 1804) were examined after single and combined exposure. Metal bioaccumulation in the digestive gland of adult individuals was investigated after 21 days exposure to 100 μM lead nitrate, 10 μM copper sulphate, and 0.002 X (1 X = 36 μM lead: 1 μM copper; a ratio matching that recorded in the snail's aquatic habitat). Lead was bioaccumulated higher than copper in the group exposed to the metal solution mixture. Elevated lead or copper concentrations were demonstrated in combined solution group relative to single metal solution examined individuals. Both metals accumulated over 50 fold in single solution examined groups and more than 800 times in combined solution tested individuals. Acute toxicity experiment showed lower 24 hour LC_{50} for snails exposed to metal mixtures rather than single solutions studied individuals. Chronic toxicity study demonstrated more histopathological damage in the digestive tubules of individuals 21 days exposed to combined metal solution relative to dissolved lead or copper examined snails. The results revealed synergistic toxic effect of both metals on *L. carinatus*. Further investigations are currently going on to examine the potential value of that snail as biomonitor for aquatic pollutants.

Key words: *Lanistes*, lead, copper, bioconcentration, toxicity levels, histopathology.

INTRODUCTION

Several authors have addressed heavy metal bioconcentration in aquatic molluscs (Sholz, 1980; Simkiss et al., 1982; Phillips & Rainbow, 1993; AbdAllah et al., 2003). The uptake of metals in freshwater bodies is a function of different variables as membrane permeability and physiological status of the organisms, pH, water temperature, water hardness, and acid radical of the metal salt. The concentration of a substance within the accumulator organism is the difference between the amount taken in and the amount released (Ravera, 2001). A mechanism operated in the digestive gland to detoxify metal pollutants, binding them with metallothionein (a sulphhydryl-rich protein with low molecular weight) or with some other agent and storing them in the lysosomes (George, 1982; Simkiss & Mason, 1983).

Heavy metal pollution has become the cause of serious concern and has attracted the attention of governmental authorities. Lasheen (1987) has described heavy metal pollution in Egypt. Generally, the ratio of heavy metals in the freshwater bodies is a function of the anthropogenic spill and natural input. However, most of the available information regarding their accumulation and toxicity were based on single metal solution experiments. Parott & Sprague (1993) showed that combination of low copper concentrations with high concentrations of zinc resulted in antagonistic effect on fathead minnows. He reported that heavy metals might interact antagonistically or synergistically depending on the type of metals and species affected. Harrahy & Clements (1997) observed that removal of zinc from a synthetic sediment contaminated with a mixture of lead, copper, zinc, and cadmium resulted in a pronounced decrease in growth and egg laying rate and an increase in the survival rate of the midge *Chironomus tentans*. Lead is considered of the most toxic heavy metals to human health, affecting nervous and excretory systems (Hutton, 1987). Also, it affects the haem synthesis mainly through inhibiting the conversion of α-aminolevulinic acid to porphobilinogen (Berry et al., 1974). Copper is a trace element needed in minute amounts by aquatic molluscs to synthesize haemocyanin (Ghiretti & Ghiretti-Magaldi, 1975; Simkiss & Mason, 1983). Increase of the copper content within the molluscan tissues resulted in toxic effects at the target organs (WHO, 1989; AbdAllah, 2000).

The toxicity studies are needed to establish the water pollution standards necessary to protect the aquatic life. Also, this kind of study can supply information about the effect of sudden discharge of pollutants on the aquatic organisms. In addition, it supplies information about their sensitivity to contaminants, determining thereby the maximum permissible concentrations for aquatic life (Clubb et al., 1975).

An extensive literature has appeared recently documenting the use of molluscs as successful sentinel organisms screening the aquatic environment for metal contaminants (Simkiss et al., 1982; Cossa, 1989; AbdAllah et al., 1999, 2003). *Lanistes carinatus* is a widely distributed gastropod in Egyptian freshwater ecosystems (Brown, 1995). It has a large enough size to provide sufficient tissue for metal analysis. Investigations concerning its storing capability of various metal pollutants, their histopathological changes, and influences on different biological activities of that species are required to set up its efficiency as biomonitor to freshwater contaminants.

The present work aims to investigate the bioaccumulation and toxicity of lead and copper for the freshwater snail *Lanistes carinatus* when examined singly and to depict the nature of toxicity of both metals together, whether synergistic or antagonistic, when mixed in a ratio resembles that in the inhabitant area. Also, the histopathological change in the digestive gland as a result of prolonged exposure to sublethal levels of these metals is described.

MATERIALS AND METHODS

Sampling

The freshwater prosobranch *Lanistes carinatus* was collected from El-Mansoureya Canal, Abou-Rawash, Giza. Before any treatment, the snails were washed in running water to remove any debris and maintained in three-liter aquaria for one week to be adapted to the laboratory conditions and to release their internal metal contents. The aquaria were aerated with electric air pumps, and the snails were fed every other day with fresh romain lettuce.

Preparation of Test Solutions

Test solutions for lead nitrate and copper sulphate were prepared in terms of molar concentrations as mentioned by AbdAllah et al. (2000). Additionally, a mixture of both metals was made in the ratio of 18 : 0.5 for lead and copper respectively, to match their observed proportion in the native habitat (18 µm lead and 0.5 µm copper).

Uptake of Lead and Copper

Groups of 30 adult and healthy snails each were exposed to 100 µM lead nitrate, 10 µM copper sulphate, and 0.002 X for 21 days in three-liter aquaria. Snails were fed fresh lettuce every other day. A space of 50 ml/snail was allowed to prevent competition of snails and to minimize the effect of snails' secretions (Thomas & Benjamin, 1974). The aquaria were continuously aerated using electric air pumps. The solutions were changed twice a week. Twenty-one days later, ten snails were collected from each group and were prepared for subsequent digestion.

Sample Preparation for Heavy Metal Analysis

The snails collected at the end of the previous experiment were crushed in a petri plate. Shell pieces were removed and the soft tissues were dissected out to isolate the digestive gland using fine scissors. The dissected organ was rinsed in pure water and weighed to the nearest 0.005 mg using a Mettler balance. Then, the excised organ was frozen at -70°C for 24 h and digested according to McDaniel (1991) and AbdAllah et al. (2003).

Determination of Heavy Metals in the Digested Tissues

Lead and copper were determined in the digested tissue using the graphite furnace spectroscopy, employing a Perkin-Elmer spectrometer with a specific-hollow cathode lamp for each metal (McDaniel, 1991; Pip, 1992; Kraak et al., 1993). The metal concentration was calculated in µg/mg wet weight.

Statistical Analysis

Two-way ANOVA followed by Student's t-test comparison of least square means were done to test the significance of metal accumulation in the different examined groups using Super-

TABLE 1. Two-way ANOVA examining the effect of lead and copper interaction on bioconcentration of metals in the digestive gland of *L. carinatus*.

Source	df	Sum of squares	Mean squares	F-value	P-value
Metal	1	3891381	13890000	16.253	0.0003
Treatment	1	38575511	38580000	45.134	0.0001
Metal*Treatment	1	13583318	13580000	15.893	0.0003
Residual	36	30768878	854691		

ANOVA software computer program, Abacus Concept, Inc., Berkeley, California. Possible correlation relationship between lead and copper levels in the digestive gland of snails exposed to snail mixture was examined. Also, regression analysis was conducted to determine the relationship between metal concentration within the digestive gland and organ weight.

Determination of Toxic Levels

Preliminary experiments were conducted to set the appropriate concentrations of each metal and the metal mixture for the toxicity studies. The lead nitrate concentrations tested were 100, 250, 500 µM, 1 mM, and 5 mM, while the examined copper sulphate concentrations were, 20, 30, 50, 100, 500, and 1,000 µM. The toxicity of lead and copper interaction was studied employing a mixture of lead nitrate and copper sulphate (1 X = 18 µM : 0.5 µM respectively). The concentrations selected for the toxicity study were 0.001 X, 0.005 X, 0.01 X, 0.05 X, and 0.1 X. Groups of ten adult healthy snails were exposed to each examined concentration for 24 h. The number of dead snails was counted. Failure to respond to needle touch was considered as sign of death. The experiment was repeated three times. LC_{25}, LC_{50}, LC_{75}, and LC_{95} were determined according to Finney (1971).

Histopathological Study of Long-Term Toxicity

The effects of chronic exposure for a period of three weeks to sublethal levels of lead nitrate (100 µM), copper sulphate (10 µM), and a mixture of these metals (0.002 X) were investigated histologically in the digestive gland. Moreover, normal histological features of control snails were described.

Following the exposure period, the exposed and control individuals were dissected and the examined organ was isolated. Paraffin blocks of that organ were prepared according to Bancroft & Stevens (1996). Five-µm thin sections were made using a rotary microtome, stained with Haematoxylin and Eosin, dehydrated in an ascending series of ethyl alcohol, cleared in xylene, and mounted in Canada balsam. The permanent preparations of digestive gland of exposed and control individuals were photographed using a 35 mm camera attached to a Zeiss light microscope.

RESULTS

Bioconcentration of Lead and Copper in *Lanistes*

Lead and copper concentrations were compared in the digestive gland of the freshwater prosobranch *Lanistes carinatus* using a two-way analysis of of variance (ANOVA) (Table 1). Significant differences ($P < 0.001$) were found between metal concentrations in the digestive glands of snails that underwent single and mixed exposure and between lead and copper levels. Also, the interaction of metal type and treatment had a significant effect on metal concentration in the examined organ ($P < 0.001$). Comparison of least square of means (Table 2) showed significant difference between lead ($P < 0.05$) or copper ($P < 0.01$) concentrations of snails exposed to single and combined metals and also demonstrated significant difference ($P < 0.01$) between capability of lead and copper bioconcentration in the digestive gland of snails exposed to metal mixture. Lead showed higher bioaccumulation factor than copper (Table 3) even in presence of mixed metals. In all cases, lead and copper are concentrated over 50 fold in the digestive gland of the snails singly exposed and more than 800 fold for snails that exposed to combined metals compared to the surrounding water. Significant negative correlation relation-

TABLE 2. Student's t-test (t-values) comparing the least square of means of lead and copper concentrations in the freshwater prosobranch *L. carinatus* after single and mixed exposure. (* $P < 0.05$, ** $P < 0.01$)

		Single exposure		Mixed exposure	
		Cu	Pb	Cu	Pb
Single exposure	Cu	–	-0.032	2.132*	ND
	Pb		–	ND	7.569**
Mixed exposure	Cu			–	-5.670**
	Pb				-

ship ($r = -0.95$) was demonstrated between lead and copper uptake in the digestive gland of snails after 21 days exposure to combined metals. Regression analysis between metal concentration and weight of the examined organ showed a significant relationship $r = -0.893$, $P < 0.05$ for lead (Fig. 1) and $r = -0.877$, $P < 0.02$ for copper (Fig. 2).

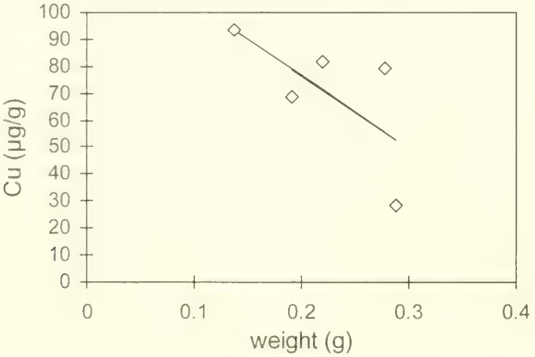

FIG. 1. Linear regression relationship between copper concentration (µg/g) and weight of the digestive gland of *L. carinatus* ($r = -0.877$, $P < 0.02$).

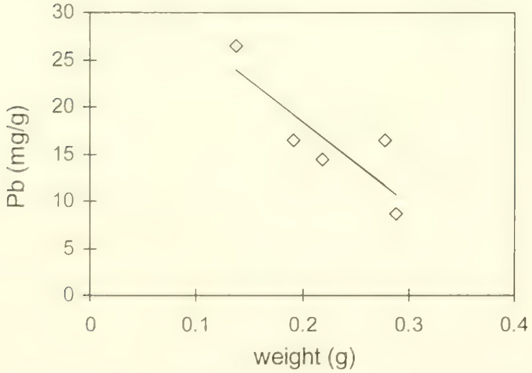

FIG. 2. Linear regression relationship between lead concentration (mg/g) and weight of the digestive gland of *L. carinatus* ($r = -0.893$, $P < 0.05$).

TABLE 3. Bioaccumulation factor of lead and copper in the digestive gland of *L. carinatus* after single and mixed long-term exposure.

	Single exposure	Mixed exposure
Copper	55.811	854.401
Lead	68.953	3198.493

Acute Toxicity of Lead and Copper

Toxicity levels: LC_{25}, LC_{50}, LC_{75}, and LC_{95} of lead, copper, and a mixture of them are demonstrated in Table 4. The data demonstrated that copper was more toxic than lead. The mixture of both metals was highly toxic relative to single metals.

Histological Structure of the Digestive Gland of *Lanistes carinatus*

The digestive gland of control snails (Fig. 3) consists of ovoid to cylindrical shaped digestive tubules. Each tubule is composed of columnar basophil cells with darkly stained granules and digestive secretory cells that exhibit the absorptive phase where the cells are partially disintegrated. These cells are rested on a basement membrane or the integument.

Histopathological Effect of Chronic Exposure to Metal Treatment

Chronic exposure to 100 µM lead nitrate (Fig. 4) resulted in presence of necrotic digestive and basophil cells in a wide tubular cavity with

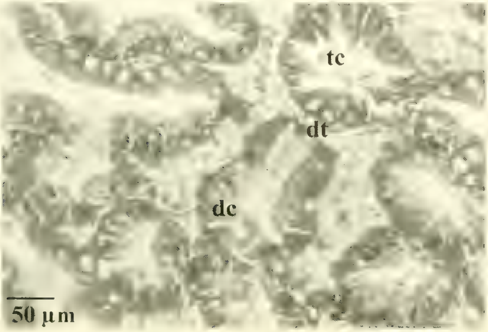

FIG. 3. Light structure of the digestive gland of *L. carinatus*; digestive cell (dc), digestive tubule (dt). Scale bar = 50 µm.

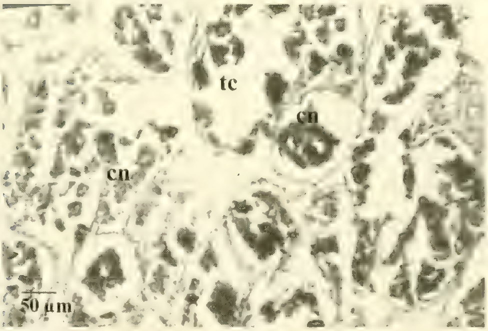

FIG. 4. Transverse section through the digestive gland of *L. carinatus* 3 weeks exposed to 100 µM lead nitrate; tubular cavity (tc), cell necrosis (cn). Scale bar = 50 µm.

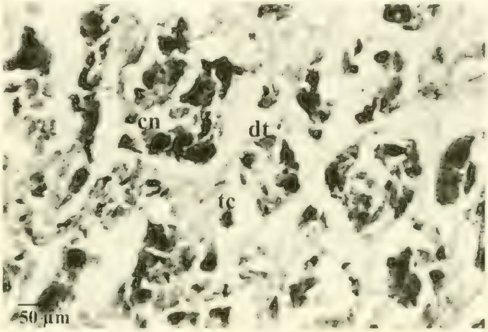

FIG. 5. Transverse section of the digestive gland of *L. carinatus* exposed to 10 µM copper sulfate for three weeks; cell necrosis (cn), digestive tubule (dt). Scale bar = 50 µm.

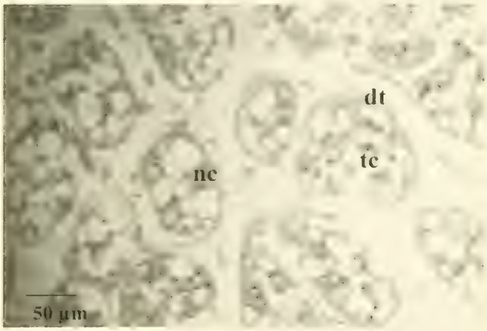

FIG. 6. Light micrograph of the cross-sectioned digestive gland excised from *L. carinatus* three weeks exposed to 0.002 X; digestive tubule (dt), tubular cavity (tc), residual necrotic cells (nc). Scale bar = 50 µm.

TABLE 4. Toxicity levels of 24 h single and combined exposure to lead, copper for the freshwater snail *L. carinatus*.

	LC_{25}	LC_{50}	LC_{75}	LC_{95}
Lead	562.34 μM	1.995 mM	6.761 mM	21.379 mM
Copper	39.81 μM	141.25 μM	251.19 μM	1.778 mM
Lead-Copper	0.0035 X	0.018 X	0.079 X	0.708 X

slight tubular deterioration. Digestive tubules of snails exposed to 10 μM copper sulphate for three weeks were almost filled with batches of damaged digestive and basophil cells. Deterioration of the digestive tubules was obvious (Fig. 5). Destructive effect as a result of three weeks exposure to a mixture of both metals (0.002 X) was clearly illustrated (Fig. 6), with the digestive tubules appearing almost vacuolated and enclosing rudiments of the disintegrated basophil and digestive cells.

DISCUSSION

The long-term toxicity data of metal pollutants can supply valuable information about the sensitivity of exposed organisms to such pollutants. In addition, the combined effect of metals is a subject worthy of study, as the metals naturally exist together in the aquatic environment in variable ratios, depending on various input sources (Clubb et al., 1975). The present investigation demonstrated a variable response of the examined prosobranch snail toward the short-term exposure to sublethal levels of lead nitrate, copper sulphate and a mixture of both metals (18 μg/l lead nitrate: 0.5 μg/l copper sulphate). The results are in agreement with the observations of Mathur et al. (1981), who found a variable acute toxicity effect of zinc, copper, and mercury on the freshwater pulmonate *Lymnaea luteola*. Recorded toxicity levels revealed that the combined effect of the two metals was more toxic than that of individual metals. This finding is in accordance with the observations of Harrahy & Clements (1997), who found that the removal of zinc from a synthetic sediment incorporated with mixture of cadmium, copper, lead, and zinc resulted in increasing the survival rate of *Chironomus tentans*. However, the results are in contrast with those of Parrott & Sprague (1993), who showed that the combination of low copper concentrations with high concentrations of zinc resulted in antagonistic effect on fathead minnows.

It is well documented that the digestive gland is the major site of metal storage in molluscs (Simkiss et al., 1982; Simkiss & Mason, 1983; AbdAllah, 1999; AbdAllah & Moustafa, 2002). A mechanism of metal detoxification was successfully operated in that organ to phagocytoze heavy metals after being chelated with a proper agent, specifically metallothionein for copper and cadmium, and carbonate or lipofucsin for lead (George, 1982; Simkiss & Mason, 1983; Philips & Rainbow, 1993). However, this mechanism has a maximal threshold, at which the toxic signs started to be manifested in that organ at higher dosages. In the present work, the uptake studies demonstrated higher capability of lead to store in the digestive gland tissues even in the presence of low copper concentrations and a significant negative correlation between lead and copper concentrations, which indicates an inversely relationship between their bioaccumulation in the gland tissues. This finding is in accordance with results of a previous study (AbdAllah & Moustafa, 2002).

Histological studies are effective as a biomarker tool indicating the pathological effect of a toxicant upon living organisms (Landis & Yu, 1995; AbdAllah, 2003). Necrosis, lesions, in addition to the appearance of disorganized, and vacuolated cell masses are the prominent features of the histopathological influence of a specific toxicant (Sullivan & Cheng, 1976; Sunila, 1984; AbdAllah, 2000). The chronic toxicity of copper and lead followed similar pattern, in which necrotic cells appeared filling the tubular cavity and being detached from the tegument in *Lanistes*. The finding is in agreement with observations of Tolba et al. (1999) for the effect of chronic exposure of the schistosome vectors *Biomphalaria alexandrina* and *Bulinus truncatus* to copper sulphate. Other studies defined the toxicity status as the increase in diameter of the digestive tubule that accompanied the reduction in cellular length (George, 1990). The effect of long-term exposure to sublethal concentrations of mixed concentrations of lead nitrate and copper sulphate on the digestive gland of *Lanistes carinatus* was more toxic compared to that shown for the single metals, with degeneration of tubular cells, expansion of the tubular cavity and detachment of tubular tegument observed. This supports the toxicity data of previous studies (Harrahy & Clements, 1997; AbdAllah et al. 2000), and indicates that the interaction of lead and copper is more toxic, compared to that of each metal singly. It is worth mentioning that the findings of this experimental study might not be valid for field investigations, because in the water canal other organic and inorganic substances are present. The interaction of these compounds with lead and copper might be antagonistic, minimizing or abolishing their toxicities. Also, the concentrations used are $^1/_{10}$ of the calculated LC_{50} and are fairly higher than that recorded in the freshwater body (18 µm lead and 0.5 µm copper). The results are also consistent with that of Wong (1987), who described this type of effect as more than an additive effect and recommended the use of metal mixtures for both chronic and acute toxicity studies rather than single metal solutions, because they supply more valuable and realistic information about the nature of heavy metal toxicities in the aquatic ecosystem. Further studies on *L. carinatus* are currently going on concerning its storage capability and sensitivity to other heavy metals and organic pollutants to investigate its potential value as biological monitor capable of screening the Egyptian freshwater ecosystem for various contaminants.

LITERATURE CITED

ABDALLAH, A. T., 1999, Heavy metal accumulation in the freshwater prosobranch *Bellamia unicolor. 10th international symposium of biomonitoring, Karlsruhe, Germany, September 28–October 1, 1999*, pp. 1–2.

ABDALLAH, A. T., 2000, On the uptake and release of copper in the freshwater snail *Bellamia unicolor. Science Journal of Al-Azhar University, Faculty of Medicine (for Girls)*, 21: 525–533.

ABDALLAH, A. T., 2003, On the effeciency of some histological techniques as biomarkers for heavy metal pollution. Pp. 287–296, in: J. S. MENDEZ, ed., *Science, technology and education of the microscopy; an overiew*, Vol. 1. Formatex Publishers, Badajoz, Spain. 436 pp.

ABDALLAH, A. T. & M. A. MOUSTAFA, 2002, Accumulation of lead and cadmium in the marine prosobranch *Nerita saxtilis*; chemical analysis, light and electron microscopy. *Environmental Pollution*, 116: 185–191.

ABDALLAH, A. T., S. N. THOMPSON, D. B. BROCHARDT & M. Q. A. WANAS, 1999, *Biompahalaria glabrata*: a laboratory model illustrating the potential of pulmonate gastropods as freshwater biomonitors of heavy metal pollutants. *Malacologia*, 41: 345–353.

ABDALLAH, A. T., A. S. BREAM & A. O. EL-MEADAWEY, 2000, Toxicity of heavy metals interaction to some freshwater invertebrates. *Al-Azhar Bulletin of Science*, 11: 9–15.

ABDALLAH, A. T., M. Q. A. WANAS & S. N. THOMPSON, 2003. Dissolved heavy metals, lead, cadmium and mercury accumulate in the body of the schistosome vector, *Biomphalaria glabrata* (Gastropoda; Pulmonata). *Journal of Molluscan Studies*, 69: 35–41.

BANCROFT, J. D. & A. STEVENS, 1996, *Theory and practice of histological techniques*, 3rd ed. Churchill Livingstone, Edinburgh & New York. 766 pp.

BERRY, J. W., D. W. OSGOOD & P. A .S. JOHN, 1974, *Chemical villains, a biology of pollution*. C. V. Mosby, Saint Louis, Missouri. vii + 189 pp.

BROWN, D. S., 1995, *Freshwater snails of Africa and their medical importance*, 2nd ed. Taylor & Francis, London. x + 608 pp.

CLUBB, R. W., A. R. GAUFIN & J. L. LORDS, 1975, Acute cadmium toxicity studies upon nine species of aquatic insects. *Environmental Research*, 9: 332–341.

COSSA, D., 1989, A review of the use of *Mytilus* spp. as quantitative indicators of cadmium and mercury contamination in coastal waters. *Oceanologia Acta*, 12: 417–432.

FINNEY, D. J., 1971, *Prohibit analysis*, 3rd ed. Cambridge University Press, London & New York. xv + 333 pp.

GEORGE, S. G., 1982, Subcellular accumulation and detoxification of metals. Pp. 3–53, in: W. B. VERNBERG, A. CALABRESE, F. P. THURBERG & F. J. VERNBERG, eds., *Physiological mechanisms of marine pollutant toxicity*. Academic Press, New York & London. xiii + 564 pp.

GEORGE, S. G., 1990, Biochemical and cytological assessments of metal toxicity in marine animals. Pp. 123–142, in: R. W. FURNESS & P. S. RAINBOW, eds., *Heavy metals in marine environment*. CRC Press, Boca Raton, Florida. 256 pp.

GHIRETTI, F. & A. GHIRETTI-MAGALDI, 1975, Respiration. Pp. 33–52, in: V. FRETTER & J. PEAK, eds., *Pulmonates*, Vol. 1. *Funcational anatomy and physiology*. Academic Press, London & New York. xxix + 417 pp.

HARRAHY, A. & W. H. CLEMENTS, 1997, Toxicity and bioaccumulation of heavy metals in *Chironomus tentans. Environmental Toxicology and Chemistry*, 16: 317–327.

HUTTON, M., 1987, Human health concerns of lead, mercury, cadmium and arsenic. Pp. 53–68, in: T. C. HUTCHINSON & K. M. MEEMS, eds., *Lead, mercury, cadmium and arsenic in the environment.* Chicester. New York. xxiii+ 360 pp. *[Scientific Committee on Problems of the Environment (SCOPE 31)].*

KRAAK, H. S., M. TOUSSAINT, A. J. BLEEKER & D. LAVY, 1993, Metal regulation in two species of freshwater bivalve. Pp. 175–186, in: R. DALLINGER & P. S. RAINBOW, eds., *Ecotoxicology of metals in invertebrates.* SETAC Special Publication Series. Lewis, Boca Raton, Florida. 461 pp.

LANDIS, W. G. & MING-HO YU, 1995, Measurement and evaluation of the ecological effects of toxicants. Pp. 197–225, in: W. G. LANDIS & MING-HO YU, *Introduction to environmental toxicology: impacts of chemicals upon ecological systems.* Lewis, Boca Raton, Florida. 328 pp.

LASHEEN, M. R., 1987, The distribution of trace metals in Aswan High Dam reservoir and River Nile ecosystems. Pp. 235–254, in: T. C. HUTCHINSON & K. M. MEEMA, eds., *Lead, mercury, cadmium and arsenic in the environment.* New York: Chicesster. xxiii + 360 pp. [*Scientific Committee on Problems of the Environment (SCOPE 31)*].

MATHUR, S., B. S. KHANGAROT & V. S. DURVE, 1981, Acute toxicity of mercury, copper and zinc to a freshwater pulmonate snail, *Lymnaea luteola* (Lamarck). *Acta Hydrochemistry and Hydrobiology*, 9: 381–389.

MCDANIEL, W., 1991, Sample preparation procedure for spectrochemical determination of total recoverable elements in biological tissues. Pp. 23–29, in: *Revision 1.0 Environmental Monitoring Systems Laboratory.* U.S. Environmental Protection Agency.

PAROTT, J. L. & J. B. SPRAGUE, 1993, Patterns in toxicity of sublethal mixtures of metals and organic chemicals determined by Microtox and by DNA, RNA and protein content of fathead minnows (*Pimephales prometas*). *Canadian Journal of Fisheries and Aquatic Science*, 50: 2245–2253.

PHILLIPS, D. J. H. & P. S. RAINBOW, 1993, *Biomonitoring of trace aquatic contaminants.* Elsevier Applied Science, New York & London. xii + 371 pp.

PIP, E., 1992, Cadmium, copper and lead in gastropods of the lower Nelson River system, Manitoba, Canada. *Journal of Molluscan Studies*, 58: 199–205.

RAVERA, O., 2001, Monitoring of the aquatic environment by species accumulator of pollutants:a review. *Journal of Limnology*, 60(Suppl. 1): 63–78.

SHOLZ, N., 1980, Accumulation, loss and molecular distribution of cadmium in *Mytilus edulis. Helgoländer Meeresuntersuchungen*, 33: 68–78.

SIMKISS, K. & A. Z. MASON, 1983, Metal ions: metabolic and toxic effects. Pp. 110–164, in: P. W. HOCHACHKA, ed., *The Mollusca*, Vol. 2. *Environmental biochemistry and physiology.* Academic Press, New York & London. xviii + 362 pp.

SIMKISS, K., M. TAYLOR & A. Z. MASON, 1982, Metal detoxification and bioaccumulation in molluscs. *Marine Biological Letters*, 3: 187–201.

SULLIVAN, J. T. & T. C. CHENG, 1976, Heavy metal toxicity to *Biomphalaria glabrata* (Mollusca: Pulmonata). *Annals New York Academy of Science*, 119: 437–444.

SUNILA, I., 1984, Copper and cadmium-induced histological changes in the mantle of *Mytilus edulis* L. (Bivalvia). *Limnologica*, 15: 523–527.

THOMAS, J. D. & M. BENJAMIN, 1974, The effect of population density on growth and reproduction of *Biomphalaria glabrata* (Say) (Gastropoda; Pulmonata). *Journal of Animal Ecology*, 43: 31–50.

TOLBA, M. R. A., M. BEDIR & M. MOSSA, 1999, Effect of Cu, Zn, Cd and Hg on the histology of the digestive gland of the freshwater snail *Biomphalaria alexandrina* and *Bulinus truncatus. Egyptian Journal of Aquatic Biology and Fisheries*, 3: 39–57.

WHO, 1989, Lead - Environmental aspects. Environmental Health Criteria, World Health Organization. Geneva. 106 pp.

WONG, P. T. S., 1987, Toxicity of cadmium to freshwater microorganisms, phytoplankton, and invertebrates. Pp. 117–138, in: J. O. NRIAGU & J. B SPRAGUE, eds., *Cadmium in the aquatic environment.* Wiley-Interscience, New York. xii + 272 pp.

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